LXXVIII. 5-NUCLEOTIDASE

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Reis [1934] described an enzyme, called by him 5-nucleotidase, which occurs in the brain of the rabbit and rat and in the retina of the frog, rat, rabbit and calf. Extracts of these tissues dephosphorylated muscle adenylic acid (adenosine-5-phosphate) and inosinic acid (inosine-5-phosphate) many times more rapidly than yeast adenylic acid (adenosine-3-phosphate) or glycerophosphate.

Subsequently, Reis [1937] showed the presence of the same enzyme in calf brain and horse nerve, and extended the list of substances which are only slowly dephosphorylated to include Embden's hexosemonophosphate, pyrophosphate, phosphoglyceric acid, phosphogluconic acid and guanine nucleotide. He attributed this unspecific hydrolysis to the action of a general phosphatase present in small amount in nervous tissue, whereas the rapid fission of adenosine (inosine)-5-phosphate is caused by 5-nucleotidase.

The position of Harden & Young's fructose-1:6-diphosphate was intermediate between that of adenosine-5-phosphate and that of the group of slightly reactive substances. Reis attributed this to the presence of the furanose ring in fructose-1:6-diphosphate, a structure also found in adenosine-5-phosphate but not in Embden's hexosemonophosphate, which is a pyranose.

In connexion with the present investigations, it should be noted that the duration of the experiments of Reis did not exceed 5 hr.

Experimental

Taking into consideration only the first few hours of the experiments of the present researches, extracts of calf retina and sheep retina resembled those described by Reis; they dephosphorylated adenosine-5-phosphate rapidly and

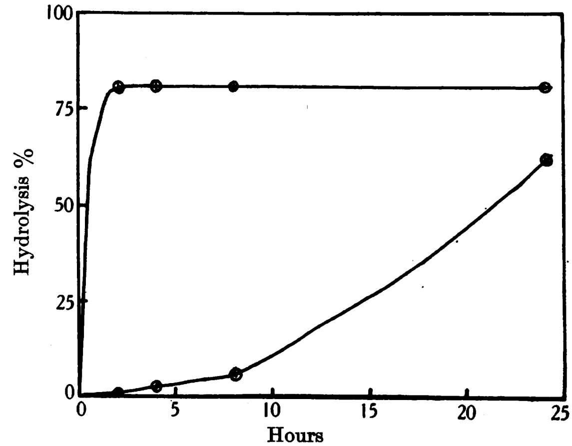

Fig. 1. Dephosphorylation of adenosine-5-phosphate and adenosine-3-phosphate by extract of calf retina under similar conditions.

had but little action on adenosine-3-phosphate. After this preliminary period, however, the rate of hydrolysis of adenosine-3-phosphate increased markedly, and at the end of 22–24 hr. the degrees of hydrolysis of the two esters were the same (Fig. 1).

The enzyme solutions were prepared from the retinae, obtained as soon as possible after death, by grinding with clean sand and chloroform-water (1 ml.: each retina) and allowing the mixture to autolyse for 24 hr. at room temperature before filtration. The filtrate is referred to as the enzyme solution. Estimations of phosphate were carried out by the Briggs's colorimetric method.

Table I. *Typical enzymic activities of extracts of calf retina and sheep retina*

(*a*) *Calf.* Yeast or muscle adenylic acid 4·2 mg., dissolved in dilute sodium carbonate; *p*H 7 veronal buffer 5 ml.; enzyme solution 2 ml.; water to 10 ml., with a drop of toluene. 37°. 2 ml. samples.

(*b*) *Sheep.* Yeast or muscle adenylic acid 5·1 mg., dissolved in dilute sodium carbonate; *p*H 7 veronal buffer 5 ml.; enzyme solution 3 ml.; water to 10 ml., with toluene. 37°. 2 ml. samples.

	Calf retina		Sheep retina	
Time hr.	Adenosine 5-phosphate hydrol. %	Adenosine 3-phosphate hydrol. %	Adenosine 5-phosphate hydrol. %	Adenosine 3-phosphate hydrol. %
0	—	—	—	—
2	81	1·8	61	5·5
4	81	3·9	—	—
5	—	—	64	44
8	81	6·5	—	—
22	—	—	100	100
24	81	62	—	—

The specific action on adenosine-5-phosphate vanished when the enzyme solutions were dialysed through No. 400 cellophane against distilled water until free from inorganic phosphate, both the 3- and 5-nucleotides being then dephosphorylated immediately and at similar rates (Table II).

Table II. *Enzymic activities of dialysed extract of sheep retina*

Yeast or muscle adenylic acid 4·6 mg., in dilute sodium carbonate; *p*H 7 veronal buffer 5 ml.; dialysed enzyme solution 3 ml.; water to 10 ml. and toluene. 37°. 2 ml. samples.

Time hr.	Adenosine-3-phosphate hydrol. %	Adenosine-5-phosphate hydrol. %
0	—	—
2	55	73
4	76	79
8	85	85

The foregoing results suggested that retina extracts contained two enzymes, 5-nucleotidase and a phosphomonoesterase capable of dephosphorylating adenosine-3-phosphate. Attempts to purify or separate the two enzymes by adsorption on norite charcoal and elution with *p*H 7·8 borate buffer or *p*H 6·0 acetate buffer were unsuccessful; the enzymes were adsorbed but were not eluted, since the eluates had no action on yeast or muscle adenylic acids.

It was evident that the tissues discussed above could not in all probability be satisfactorily used for 5-nucleotidase preparations, and it was therefore decided to investigate other possible sources of this enzyme.

Recently, it was shown [Gulland & Jackson, 1938] that the venoms of Russell's viper (*Vipera russellii*), the water mocassin (*Agkistrodon piscivorus*), the banded krait (*Bungarus fasciatus*) and the diamond rattlesnake (*Crotalus adamanteus*) contain a diesterase, which liberates phenol from diphenylphosphate, but do not contain a monoesterase which can liberate inorganic phosphate from monophenylphosphate. It has now been observed that these venoms rapidly dephosphorylate adenosine-5-phosphate and inosine-5-phosphate [Embden, 1929] (Table III) but have no action whatsoever on adenosine-3-phosphate or α- or β-glycerophosphate. Further, it has been shown that Russell's viper venom entirely fails to liberate phosphate from guanylic acid, cytidylic acid, glucose-6-phosphate, fructose-6-phosphate, mannose-6-phosphate, trehalose monophosphate and fructose-1:6-diphosphate.

Table III. *Enzymic activities of the venoms of Russell's viper, water mocassin, banded krait and diamond rattlesnake*

Adenosine-5-phosphate 7·0 mg. in a little dilute sodium carbonate, or barium inosinate 7·0 mg., converted into sodium salt by means of sodium sulphate; 10 mg. venom* dispersed by grinding in water; pH 8·6 borate buffer 5 ml.; water to 10 ml. and some chloroform. 37°. 2 ml. samples.

	Russell's viper 5-phosphate of		Water mocassin 5-phosphate of		Banded krait	Diamond rattlesnake
Time hr.	Adenosine hydrol. %	Inosine hydrol. %	Adenosine hydrol. %	Inosine hydrol. %	Adenosine-5-phosphate hydrol. %	Adenosine-5-phosphate hydrol. %
0	22·0†	30†	32†	25†	9†	18†
2	74	58	70	58	—	—
3	—	—	—	—	64	70
4	—	61	—	63	—	—
6	76	—	70	—	—	—
24	79	—	75	—	70	72

These venoms did not liberate inorganic phosphate in 24 hr. from any of the other substrates mentioned in the text.

* The venoms, extracted from living snakes, were dried in a vacuum at 35° immediately after extraction. They were then hermetically sealed and stored in the dark.

† Since neither the venoms nor the substrates contained inorganic phosphate, this hydrolysis had occurred before the sample could be taken.

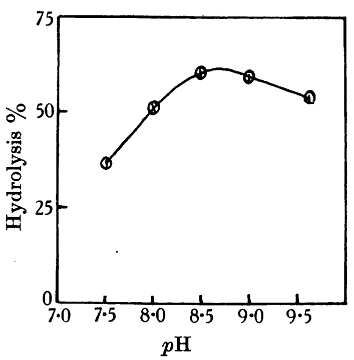

Fig. 2. pH-activity curve of 5-nucleotidase in Russell's viper venom. Adenosine-5-phosphate 1·51 mg. in 2 ml. water; venom 3 mg. dispersed in 2 ml. water; veronal or borate buffer 5 ml.; water to 10 ml. 37°. Time 30 min. 4 ml. samples.

It is evident that the venoms of Russell's viper, water mocassin, banded krait and diamond rattlesnake are rich in 5-nucleotidase, and in the first case the enzyme is active over a comparatively wide pH range, with a slight optimum at pH 8·5–9 (Fig. 2).

Discussion

The conclusions drawn by Reis from his experiments with brain, nerve and retina were that these tissues contain an enzyme, 5-nucleotidase, which is specific for adenosine(inosine)-5-phosphate, and that this enzyme is accompanied by small amounts of a non-specific phosphomonoesterase which, on account of its low concentration, effects only slight hydrolysis of other phosphoric esters, one of which is adenosine-3-phosphate. In the present investigation, these results have been confirmed as regards retinae when the experiments were of relatively short duration. It has now been shown, however, that if the experiments are more prolonged dephosphorylation of adenosine-3-phosphate occurs rapidly after an initial period of but slight hydrolysis. Moreover, it has been found that the degree of hydrolysis of adenosine-3-phosphate reaches that of adenosine-5-phosphate. The explanations put forward by Reis for the facts observed by him and hitherto sufficient now seem to require modification in the light of the new results, and in retinae there seems to be, at any rate potentially, a considerable amount of an enzyme which can dephosphorylate adenosine-3-phosphate.

The reasons for the time-lag in the hydrolysis of adenosine-3-phosphate are not clear. The shape of the hydrolysis curve makes it improbable that the lag is the effect of two existing enzymes acting in conjunction and causing, for example, a transfer of the phosphoric acid residue from the 3- to the 5-position followed by hydrolysis by the 5-nucleotidase, a change which would be analogous to the enzymic conversion of 3-phosphoglyceric acid into pyruvic acid by way of 2-phosphoglyceric acid [Lohmann & Meyerhof, 1934; Meyerhof & Kiessling, 1935]. The lag may be due to a delayed development or activation of the non-specific phosphomonoesterase which hydrolyses adenosine-3-phosphate, or to the development of an activator which enables 5-nucleotidase to attack this substrate. Or, finally, the lag may be the result of the gradual inactivation of an inhibitor which restrains the action of an already existing phosphomonoesterase. This last alternative may receive some experimental support from the fact that dialysis of the retina extract so changes its specificity that hydrolyses of both adenosine-3- and -5-phosphates occur immediately and at similar rates; possibly the inhibitor passes out through the dialysing membrane.

It is evident that the venoms of Russell's viper, the water mocassin, the banded krait and the diamond rattlesnake are less contaminated sources of 5-nucleotidase than brain, retina and nerve; these venoms very actively dephosphorylate adenosine-5-phosphate and inosine-5-phosphate, and although they contain a diesterase, they are entirely free from a non-specific phosphomonoesterase; one of them has no action on any of the eleven other phosphoric esters which were investigated, whilst the others behave similarly but have not been so fully investigated.

Reis observed that preparations from brain and nerve had considerable dephosphorylating action on fructose-1:6-diphosphate and attributed this hydrolysis to the presence of the furanose structure in this ester. Since the venom of Russell's viper has no dephosphorylating action whatsoever on fructose-1:6-diphosphate, it seems more probable that the hydrolysis observed by Reis was the result either of the action of a specific enzyme or of an increased susceptibility of this ester to the action of the non-specific phosphomonoesterase as compared with that of the group of other phosphoric esters which were but slightly attacked.

It should be noticed that the venoms which have been tested for 5-nucleotidase activity have all been found to contain this enzyme. Unfortunately it has not been possible to examine for its presence those venoms which contain a non-specific phosphomonoesterase [Gulland & Jackson, 1938], because the dephosphorylating action of the non-specific enzyme would mask the effect of the specific esterase. It is hard, however, to avoid the speculation that the presence of the active 5-nucleotidase, with its specific effect on two nucleotides which play important parts in vital processes, may be related to some of the toxic effects of snake venoms.

Summary

1. Extracts of calf and sheep retinae contain specific 5-nucleotidase, and during the early part of experiments rapidly dephosphorylate adenosine-5-phosphate but have only slight action on adenosine-3-phosphate.

2. After an initial period in which the above conditions hold, dephosphorylation of adenosine-3-phosphate occurs rapidly, and soon the degrees of hydrolysis of the 3- and 5-nucleotides become similar.

3. Dialysis so alters retina extract that the specificity for adenosine-5-phosphate is lost and both 3- and 5-nucleotides are rapidly dephosphorylated immediately and at similar rates.

4. It is concluded that retina extract contains considerable amounts of a phosphomonoesterase which, under suitable conditions, can dephosphorylate adenosine-3-phosphate. This view is contrary to the conclusion of Reis.

5. The venoms of Russell's viper, the water mocassin, the banded krait and the diamond rattlesnake, are rich and specific sources of 5-nucleotidase, since they rapidly dephosphorylate adenosine-5-phosphate and inosine-5-phosphate but have no action whatsoever on monophenylphosphate, α- or β-glycerophosphate and adenosine-3-phosphate. Russell's viper venom also failed completely to dephosphorylate seven other monophosphoric esters.

6. Taking into consideration the experiments of Reis, 5-nucleotidase dephosphorylates specifically adenosine-5-phosphate and inosine-5-phosphate but does not attack fifteen other monophosphoric esters.

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